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Significance of xylem water conductance for the compatibility of maté phenotypes (*Ilex paraguarensis*) for grafting

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Summary

In this study the compatibility for grafting of root stocks and buds of three recognized leaf phenotypes of *Ilex paraguarensis* St. (maté) Hil. from south Brazil was investigated. Special regard was given to the anatomical structure and the sap flow in the secondary xylem of the grafted plants. Significant differences of the wood structure expressed in terms of vessel portion, vessel member length, and intervessel perforations were found in low compatible phenotypes, while highly compatible phenotypes had a very similar wood structure. In grafts of highly compatible phenotypes six months after grafting the water conducting system of root stocks and buds was strongly linked to each other, while in grafts of low compatible phenotypes the formation of connecting vessel elements was rare. In the first month after grafting the xylem sap flow in the buds of all grafts was significantly higher compared to their root stocks, while in the second month in grafts of highly compatible phenotypes, the water transport in the root stocks and in the buds was balanced. In contrast, in grafts of low compatible phenotypes even in the second month the water transport in the root stocks was lower compared to the buds causing the dieback of a high portion of these plants.

Introduction

Maté-tea produced from leaves and young branches of *Ilex paraguariensis* St. Hil. (*Aquifoliaceae*) is an important non-wood forest product in South Brazil as well as in parts of Argentina, Paraguay, and Uruguay. Due to its content of caffeine and theobromine, in this region the consumption of maté tea is similar to the consumption of coffee or black tea in other regions of the world. For the production of health and wellness teas, considerable amounts of maté are also exported to Asia, Europe, and North America (OHEM and HÖLZL, 1990).

Depending on the origin of the seeds, the age of the plants, and the sampling time the extractive content and the flavour of the leaves strongly vary (KAWAKANI and KOBAYASHI, 1991; MARTINEZ et al., 1997; VALDUGA et al., 1997; SCHERER et al., 2002). In spite of the high variability within the species breeding programs for the improvement of the quality and the quantity of maté production are rare. So far, in practice locally known varieties of leaf phenotypes (shape and colour of the leaves) are used for seed propagation, while vegetative propagation is only used in smaller scales (ROSSE and FERNANDES, 2002).

Ecological studies and investigations on the chemical composition of the leaves showed significant differences between leaf phenotypes with regard to their drought tolerance and the flavour of the final product (BAAS, 1973; 1975; REISSMANN et al. 1999). Consequently, with regard to the ecological stability of maté plants under different site conditions and high quality maté production appropriate combinations of root stocks and buds are desired.

In former studies REISSMANN et al. (2003) and DÜNISCH et al. (2004) found a significant correlation between the wood structure in the secondary xylem, the morphology of the leaves, and the adaptation to drier sites in different leaf phenotypes from South Brazil. It turned out that ecologically best adapted phenotypes did not produce the best quality of maté, while the phenotypes, which produced the best quality of maté were more sensitive to drought conditions. Therefore in this study the compatibility of root stocks and buds of three recognized leaf phenotypes of *Ilex paraguarensis* from south Brazil for grafting was investigated. Special regard was given to the anatomical structure and the sap flow in the xylem of the grafted plants.

Material and methods

Plant material and grafting procedure

One hundred and fifty plants each of the maté phenotypes “Amarelinha”, “Cinza”, and “Sassafras” of *Ilex paraguariensis* St. Hil. (detailed description of the phenotypes by REISSMANN et al., 2003) were obtained by vegetative propagation from five 15-year-old trees each grown at the Fazenda Bitumirim in the state of Paraná, Brazil (25°15'S, 50°45'W, 870 m above sea level; annual precipitation 1,570 mm, mean air temperature 19.1 °C). For root formation the cuttings were cultivated in a “Knop” nutrient solution for two months. After that the plants were transferred into pots with a standard soil substrate (DÜNISCH et al., 2006) and cultivated in the greenhouse of the Fazenda Bitumirim under subtropical climate conditions.

After two years of growth grafts with all nine possible combinations of root stocks and buds of the three phenotypes were produced (50 plants per treatment). For copulation grafting cuttings for roots stocks and buds were produced with sterile cutter knives at approximately 100 mm plant height. Before grafting the cut fuses of the two internodes were disinfected with 0.5 % Chinisol. The connecting zone was covered with grafting wax (Lauril, Germany) and sealed with parafilm. During a six month period the survival rate of the grafts was monitored weekly.

Microscopical characteristics of the xylem

Six months after grafting samples from the root stock, the grafting zone, and the bud of three plants of each treatment were collected. After harvest the samples were fixed in a formalin/acetic acid/alcohol (FAA) solution. The relevant shoot portions were embedded in polyethylene glycol (PEG 1500) with increasing concentration (PEG 1500:H₂O, 1:2, 1:1, 2:1, 1:0, 1:0). Transverse, radial, and tangential sections were prepared with a sliding microtome (Reichert, Austria; section thickness: 15 µm). The sections were mounted on glass slides in drops of a 25 % solution of NH₄OH (WATANABE et al., 2004). The sections were covered with coverslips and were analysed using a light microscope (Axioskop MC 80 Zeiss, Germany). Microphotos were taken with a digital camera adjusted on the microscope.

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The cross sections of the root stocks and the buds were used to determine the percentage of cell type and the radial and tangential diameter of the vessels. The percentage of cell type was obtained by means of an integration ocular lens (Leucodiff; HÖSTER and SPRING, 1971). For the quantification of the percentage of cell type 400 spots each per increment zone were analysed. The diameter of the vessels was measured directly in the microscope with an eye piece micrometer (50 measurements per treatment). The number of bars per vessel perforation plate was counted in the radial sections.

The length of the vessel members was determined after maceration in a Jeffrey's solution. The length of 60 vessel members was measured in each fraction by means of an image analyzer (SigmaScanPro).

The mean value and the standard deviation of each parameter were calculated. The significance of differences between phenotypes was assessed by ANOVA at $p \leq 0.05$ by Fisher's F-test.

Staining of the water conducting xylem and calculation of hydraulic conductivity

The water conducting system of the secondary xylem was marked using 1% methylenblue as a tracer (Fig. 1). Shoot samples (100 mm length) of five plants each of the phenotypes "Amarelinha", "Cinza", and "Sassafras" were cut under water to avoid air embolism. Dye was introduced into the samples via the transversal cut with a pressure of 10 cm water column for 5 minutes (DÜNISCH and MORAIS, 2002). Staining of the samples was quantified microscopically with regard to stained and unstained cell types and the maximum distance of stained cells from the transversal cut, where dye was applied. The hydraulic conductivity of different cell types was calculated as the portion of the cross section areas of stained cells in different distances from the transversal cut (2 mm steps).



Fig. 1: Staining of the water conducting xylem in the stem of a three-year-old maté plant (phenotype "Amarelinha") with methylene blue. Scale bar = 2 mm.

Xylem sap flow measurements

Xylem sap flow measurements were carried out according to GRANIER (1985; 1987) with a constant heating method using heated and unheated thermocouples (UP GmbH, Osnabrück, Germany). Probes were installed 30 mm below (root stock) and 30 mm above (bud) the grafting zone. The upper sensor was continuously heated with a constant power supply (156 mW). Data were recorded continuously in 10-minute averages on Skye Datahog dataloggers (Skye Instruments, Wales, U.K.).

In order to calculate the xylem sap flow from the temperature gradients measured with the thermocouples, the measuring system was calibrated by correlating temperature gradients with the water flux (sap volume) through 100 mm shoot pieces of the three pheno-

types (ERBREICH, 1997). For all three phenotypes a similar relationship was found, close to the relationship described by GRANIER (1985; 1987).

Results

Survival rate of grafted maté plants

Six months after grafting the survival rate of the grafts varied between 6 % and 94 % (Tab. 1). The survival rate of grafts produced from root stocks and buds of the same morphotype was between 82 % and 94 %. Except in plants with root stocks of the morphotype "Sassafras", the survival rate of grafts produced from root stocks and buds of different leaf phenotypes was strongly reduced. In particular the survival rate of grafts with root stocks of the morphotype "Cinza" and buds of the morphotype "Amarelinha" was extremely low (6 % corresponding to 3 plants). In these grafts 39 out of the 50 scions died in the first three months of the experiment.

Tab. 1: Survival rate (%) of grafted maté plants produced from different combinations of root stocks and buds of the phenotypes "Amarelinha", "Cinza", and "Sassafras" six months after grafting. 50 plants per treatment.

Morphotype		Buds		
		Amarelinha	Cinza	Sassafras
Root stocks	Amarelinha	82	38	34
	Cinza	6	94	62
	Sassafras	80	84	90

Anatomical characteristics of the secondary xylem of the grafted maté plants

In the secondary xylem of the grafts vessels, fibre tracheids, axial and ray parenchyma were present. In the xylem formed before grafting the distribution of the xylem elements was diffuse. No significant differences of the vessel diameter and the percentile portion of fibre tracheids were found between the three phenotypes (Tab. 2). In contrast the portion of vessels was significantly higher in the xylem of the phenotypes "Amarelinha" and "Sassafras" compared to the phenotype "Cinza". The overlap in class size distribution of measured vessel portions between the phenotypes "Amarelinha" and "Cinza" was 0 %, between "Amarelinha" and "Sassafras" 87 %, and between "Cinza" and "Sassafras" 1 % (Fig. 2). Significant differences in the length of the vessel members and the number of bars in their scalariform perforation plates were found between the three phenotypes. The longest vessel members and the highest number of vessel bars were found in the xylem of the morphotype "Amarelinha", while the shortest vessel members and the lowest number of vessel bars were found in the xylem of the phenotype "Cinza". The overlap in class size distribution of the length of vessel members between the phenotypes "Amarelinha" and "Cinza" was 29 %, between "Amarelinha" and "Sassafras" 61 %, and between "Cinza" and "Sassafras" 63 % (Fig. 3). The overlap in class size distribution of counted vessel bars between the phenotypes "Amarelinha" and "Cinza" was 0 %, while the overlap between "Sassafras" and "Cinza" and "Sassafras" and "Amarelinha" was 14 % and 5 %, respectively (Fig. 4).

Six months after grafting in the connecting zone of best compatible phenotypes (high survival rate, Table 1) the xylem tissue of root stocks and buds was strongly linked to each other in particular by the formation of connecting vessel elements and fibre tracheids

Tab. 2: Anatomical characteristics of the water conducting system and relative hydraulic conductivity in the xylem of the phenotypes “Amarelinha”, “Cinza”, and “Sassafras”. Mean value \pm standard deviation. Values followed by different letters differ significantly at $p < 0.05$ (Fisher’s F -test).

Characteristics	Amarelinha	Cinza	Sassafras
Vessel portion (%)	28 \pm 7 ^a	16 \pm 4 ^b	26 \pm 2 ^a
Vessel diameter (μm)	78 \pm 15 ^a	79 \pm 17 ^a	74 \pm 18 ^a
Vessel member length (μm)	832 \pm 132 ^a	499 \pm 98 ^b	698 \pm 112 ^c
Number of vessel bars per perforation plate	23 \pm 4 ^a	15 \pm 3 ^b	20 \pm 3 ^c
Fibre tracheids portion (%)	43 \pm 5 ^a	47 \pm 5 ^a	42 \pm 2 ^a
Relative hydraulic conductivity vessels (%)	100	34	73
Relative hydraulic conductivity fibre tracheids (%)	2	8	3

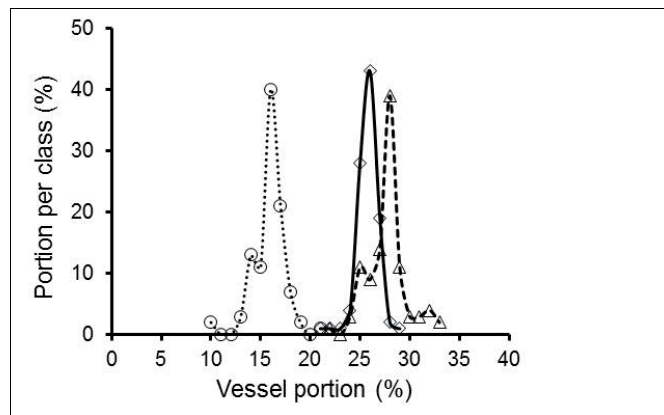


Fig. 2: Class size distribution (%) of vessel portions (%) in the xylem of the phenotypes “Amarelinha” (rough dotted line), “Cinza” (fine dotted line), and “Sassafras” (full line). Class size: 1 %. Measuring area: 1 mm².

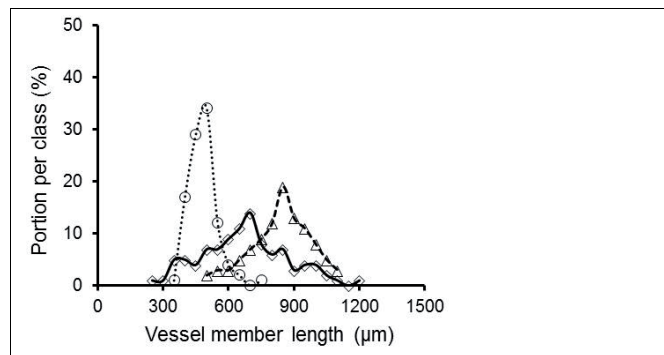


Fig. 3: Class size distribution (%) of vessel member length (μm) in the xylem of the phenotypes “Amarelinha” (rough dotted line), “Cinza” (fine dotted line), and “Sassafras” (full line). Class size: 50 μm . 1500 measurements per phenotype.

(Fig. 5a). In grafts of less compatible combinations of root stocks and buds the formation of connecting vessels and fibre tracheids was strongly reduced. In these cases the formation of a protective layer of parenchyma cells with dark staining compounds within was observed

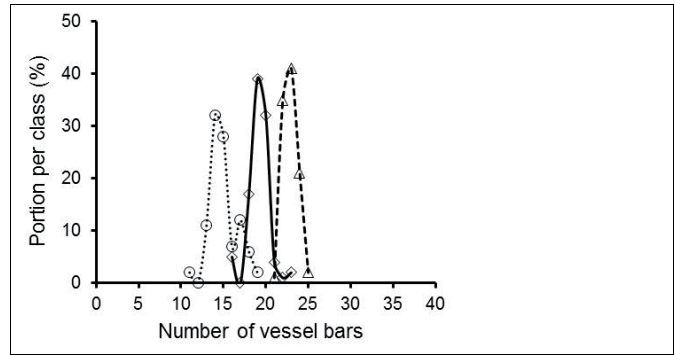


Fig. 4: Class size distribution (%) of the number of vessel bars per perforation plate in the xylem of the phenotypes “Amarelinha” (rough dotted line), “Cinza” (fine dotted line), and “Sassafras” (full line). Class size: 1. 500 measurements per phenotype.

(Fig. 5b/c). The lowest formation of connecting vessel elements and fibre tracheids and the strongest formation of a protective layer were found in root stocks of the phenotype “Cinza” connected to buds of the phenotype “Amarelinha” (Fig. 5c).

Water transport in the secondary xylem of the grafted maté plants

The transport of water in the secondary xylem of the two-years-old plants was equally distributed over the shoot cross section (Fig. 1). Water transport was restricted to vessels and fibre tracheids. Due to the very low hydraulic conductivity of fibre tracheids compared to vessels (Tab. 2), in the shoot of all phenotypes the predominant portion of water was transported in the vessels. However, the hydraulic conductivity of the vessels in the xylem of the morphotype “Cinza” was strongly reduced compared to the phenotypes “Amarelinha” and “Sassafras” (reduction 66 % and 39 %, respectively).

During the first month after grafting significant differences in xylem sap flow between the root stocks and the buds of the grafts were found (Tab. 3a). In all grafts xylem sap flow was significantly higher in the buds compared to the root stocks. Highest differences in xylem sap flow between root stocks and buds were found in grafts with root stocks of the phenotype “Cinza” and buds of the phenotype “Amarelinha” (0.004 l cm⁻² day⁻¹ and 0.039 l cm⁻² day⁻¹) corresponding to a water loss of the buds of approximately 0.53 l during the first month.

Except in grafts of combinations of root stocks and buds of the phenotypes “Cinza” and “Amarelinha”, “Amarelinha” and “Cinza”, and “Amarelinha” and “Sassafras” in the second month after grafting the xylem sap flow of root stocks and buds of all grafts was balanced (Tab. 3b). The strong differences of xylem sap flow between the root stocks and buds in “Cinza-Amarelinha”, “Amarelinha-Cinza”, and “Amarelinha-Sassafras” – grafts even in the second month after grafting corresponded to the low survival rate of these combinations of root stocks and scions (Tab. 1).

Discussion

In terms of leaf morphology, shoot anatomy, and chemical composition, the variability within the species *Ilex paraguariensis* St. Hil. (maté plant) is very high (BAAS, 1973; 1975; REISSMANN et al., 1999). Therefore over decades best cultivars were selected with regard to their ecological stability and the quality of the final product. In total, six to nine distinct varieties of *Ilex paraguariensis* are

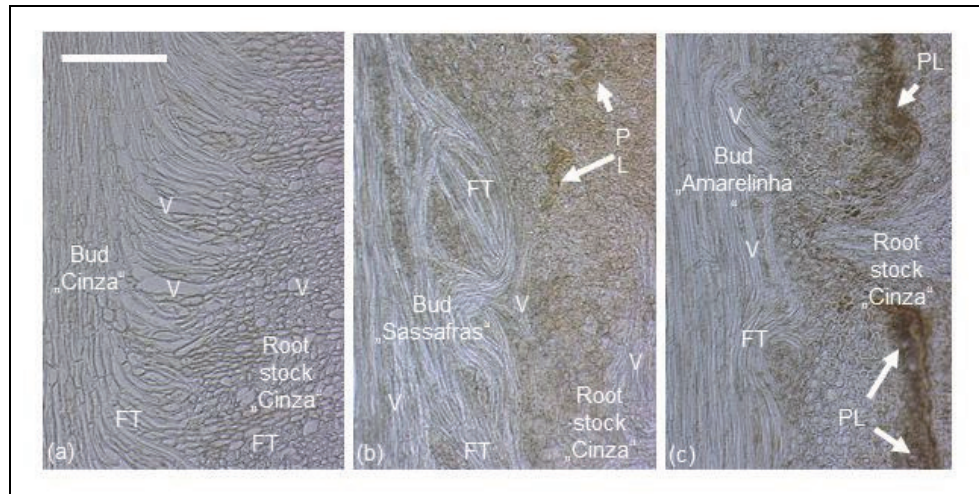


Fig. 5a-c: Wood structure of the connecting zone between the root stock of the phenotype “Cinza” and the bud of (a) the phenotype “Cinza”, (b) the phenotype “Sassafras”, and (c) the phenotype “Amarelinha” six months after grafting. FT = fibre tracheids, PL = protective layer, V = vessel. Scale bar = 200 μm .

Tab. 3: Xylem sap flow ($1 \text{ cm}^{-2} \text{ day}^{-1}$) in the root stocks and in the buds of grafted maté plants produced from different combinations of root stocks and buds of the phenotypes “Amarelinha”, “Cinza”, and “Sassafras” in the (a) first and in the (b) second month after grafting. Five plants per treatment.

(a)		Buds					
		Amarelinha		Cinza		Sassafras	
		Root stock	Bud	Root stock	Bud	Root stock	Bud
Root stocks	Amarelinha	0.012±0.004	0.043±0.009	0.007±0.003	0.023±0.009	0.009±0.006	0.029±0.008
	Cinza	0.004±0.001	0.039±0.010	0.002±0.001	0.025±0.007	0.005±0.002	0.024±0.011
	Sassafras	0.007±0.003	0.032±0.011	0.003±0.001	0.030±0.011	0.008±0.002	0.031±0.010
(b)		Buds					
		Amarelinha		Cinza		Sassafras	
		Root stock	Bud	Root stock	Bud	Root stock	Bud
Root stocks	Amarelinha	0.060±0.003	0.062±0.005	0.011±0.004	0.017±0.006	0.017±0.005	0.028±0.006
	Cinza	0.009±0.002	0.022±0.003	0.036±0.006	0.037±0.007	0.022±0.005	0.029±0.010
	Sassafras	0.061±0.009	0.057±0.004	0.032±0.003	0.029±0.003	0.049±0.008	0.048±0.007

described in the literature (COELHO et al., 2002). Inheritance studies indicate a high genetic variability between, but a smaller genetic variability within the varieties (SCHERER et al., 2002). Consequently, the genetic variation within the phenotypes selected for our experiment is supposed to be low, although the grafts were produced from five – genetically not identical - plants of each phenotype. The high survival rate of grafts produced from root stocks and buds of identical phenotypes (blanc samples) proves that an appropriate grafting procedure for the production of the experimental plants was chosen.

Studies of MOORE (1983), WANG and KOLLMANN (1996), and ESPEN et al. (2005) underline the significance of the restoration of the continuity of the vascular tissue for the compatibility of root stocks and buds in grafts. Our tracer experiments showed that in the secondary xylem of *Ilex paraguariensis*, vessels as well as fibre tracheids make part of the water conducting vascular tissue. However, in the three phenotypes the hydraulic conductivity of the vessel system was 4 to 50 times higher than in the tissue of fibre tracheids. In agreement with investigations of BECKER et al. (1999)

this result confirms the extraordinary importance of the vessel system for the water transport in the shoot.

In terms of vessel portion, vessel member length and perforations, significant differences between the three phenotypes were found, while the anatomical structure and the portion of fibre tracheids of the three phenotypes were very similar. A low portion of short vessels with a reduced number of perforations reduce significantly the maximum capacity of water transport in the xylem (TYREE and ZIMMERMANN, 2002). In contrast, bigger and longer vessels are more susceptible to air embolism under drought conditions (SPERRY and IKEDA, 1997). Consequently, the water conducting system of the phenotype “Amarelinha” could be considered to be the most effective, but most vulnerable, while the water conducting system of the phenotype “Cinza” is supposed to be the least effective, but the most resistant to drier periods of the 3 morphotypes. The distribution of the anatomical characteristics of the vessel system in the phenotype “Sassafras” had a strong overlap to the vessel system of the phenotype “Amarelinha” as well as to the vessel system of the phenotype “Cinza”. This explains the high survival rate of grafts with

root stocks or buds of the phenotype “Sassafras”. Due to the lower quality of the leaves for maté production and the low vulnerability of the water conducting system under drought conditions, the phenotype “Sassafras” seems to be most suitable for the production of universal root stocks in grafts. In addition, the results also show that vessel member length is the best wood anatomical indicator for the prediction of the compatibility of cuttings from different maté varieties in grafts.

In highly compatible grafts the water cohesion between root stocks and buds was restored by the formation of a high number of connecting vessel elements. This requires almost identical vessel characteristics in the root stock and the bud. In low compatible grafts (e.g. phenotypes “Amarelinha”/“Cinza”) only fibre tracheids (no significant differences between phenotypes) are suitable connecting elements for the restoration of the water transport between the root stocks and the scions. However, due to the lower water conductivity of fibre tracheids compared to vessels the buds of these grafts suffer from a restricted water supply (TYREE and EWERS, 1991; TYREE and ZIMMERMANN, 2002). This was confirmed by the xylem sap flow measurements below and above the grafting zone, indicating a high transpiration of the buds and a very low water flux from the root stock to the scion. According to SHIGO (1984) the observed formation of a “protective layer” in the root stock of incompatible grafts is considered to compartmentalize the root stock from the incompatible bud favoring the chance for the formation of coppice shoots in the root stocks.

The balanced water flux in the root stocks and the buds of compatible grafts in the second month of growth indicates that in these grafts the restoration of the water conducting system of the secondary xylem is very fast and lasts between a couple of days and 5 weeks as a maximum.

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